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CITATION:

Muto, Nozomu ...[et al]. Distinct genetic isolation between "Kunimasu" (Oncorhynchus kawamurae) and "Himemasu" (O. nerka) in Lake Saiko, Yamanashi Prefecture, Japan, inferred from microsatellite analysis. Ichthyological Research 2013, 60(2): 188-194

ISSUE DATE:

2013-04

URL:

http://hdl.handle.net/2433/187150

Restricted gene flow between “Kunimasu” (*Oncorhynchus kawamurae*) and “Himemasu” (*O. nerka*) in Lake Saiko, Yamanashi Prefecture, Japan, inferred from microsatellite analysis

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Short running head: Restricted gene flow of *Oncorhynchus kawamurae*

Full paper

17 text pages

Two figures, two tables, one Electronic Supplementary figure and two ES tables

Abstract

Introgressive hybridization between Kunimasu (*Oncorhynchus kawamurae*) and Himemasu (*O. nerka*) was investigated in a large number of specimens, in a search for basic data relevant to conservation needs of the former. A Bayesian-based clustering method using five microsatellite DNA loci separated 144 specimens from Lake Saiko, Yamanashi Prefecture, into two genetically distinct groups, corresponding to Kunimasu and Himemasu. Application of a threshold of individual proportion of membership (*q*i) of 0.90, so as to separate hybrids from purebreds, resulted in only two specimens with *q*i < 0.90 (therefore identified as hybrids), the remaining specimens all having *q*i > 0.90 (indicating high purebred probability). The dominant Kunimasu genome in both of the hybrid specimens indicated that they were not F1 progeny, the overall results implying that any introgressive hybridization between Kunimasu and Himemasu has been highly restricted. A second Bayesian clustering analysis demonstrated the absence of hybrids with either a Kunimasu or Himemasu ancestor within two generations. An estimate of the contemporary genetic diversity of Kunimasu showed that species to have retained a relatively high genetic diversity compared to Himemasu and other kokanee populations. Both the limited introgressive hybridization with Himemasu and high genetic diversity indicated that the present Kunimasu population in Lake Saiko should not be considered to be seriously endangered, at least on the basis of its genetic characteristics.

Keywords *Oncorhynchus kawamurae* · *Oncorhynchus nerka* · Introgressive hybridization · Genetic diversity · Microsatellite DNA

Introduction

Oncorhynchus kawamurae Jordan and McGregor *in* Jordan and Hubbs (1925) (common name "Kunimasu") had been believed extinct in Lake Tazawa, the only known habitat of the species, since 1940, due to the introduction of acidic water to the Lake from the Tama River (Sugiyama 2000). Although eyed-eggs of Kunimasu had been introduced to several other lakes, including Lake Saiko, Yamanashi Prefecture, before 1940, the lack of subsequent records of Kunimasu from these lakes had led to a belief that the species had become extinct. Accordingly, Nakabo et al's. (2011) recent discovery of Kunimasu in Lake Saiko attracted a great deal of attention concerning the conservation of the species.

Introgressive hybridization, by which process genes are exchanged between distinct species, has often been observed between native and introduced salmonid species, including some lineages that have been isolated for more than a million years (Utter 2001), and is therefore regarded as a major threat to the persistence of native evolutionary lines. Although Nakabo et al. (2011) rejected the likelihood of extensive introgressive hybridization between Kunimasu and co-occurring kokanee (land-locked sockeye salmon, *Oncorhynchus nerka*; common name "Himemasu") on the basis of clear morphological and genetic differences, the possibility of a low level of introgressive hybridization was not fully explored due to the small Kunimasu sample.

Because the identification of potential risks for a population under threat is essential for appropriate conservation measures, the presence of introgressive hybridization between Kunimasu and Himemasu was assessed, based on variations in five microsatellite loci. A large number of specimens collected from Lake Saiko throughout 2010–2011, including some of equivocal body coloration, overcame the problem of insufficient sample. Contemporary genetic diversity of Kunimasu was also determined for comparison with Himemasu populations from several other Japanese lakes, in addition to some North American kokanee populations. Because the extant population of Kunimasu in Lake Saiko is most likely to have resulted from the ca.

100,000 eyed eggs introduced from Lake Tazawa 70 years BP (Sugiyama 2000), the possibility of any recent bottleneck having occurred in the Kunimasu population, thereby reducing genetic diversity, was also investigated, using a suite of measures.

The relationships of Kunimasu and *O. nerka* are also briefly discussed on the basis of genetic divergence between them.

Materials and Methods

Sampling. A total of 115 specimens collected from Lake Saiko in 2010 and 2011 by gill net and angling, together with additional "beached" post-spawning ("Uki-yo") specimens, were deposited in the Fish Collection of Kyoto University Museum (FAKU) and the Yamanashi Prefectural Fisheries Technology Center (YFTC) (Table S1). Each specimen was then provisionally identified for the genetic analysis on the basis of external morphology (body coloration) and collection date. On the basis of Nakabo et al's (2011) observation that Kunimasu specimens collected during or just after spawning (in March and April) had a black body, 24 of the present specimens having a black body and collected from the presumed spawning ground in March and April (FAKU 98834–98847, YFTC 14, 15, 18–20, 22–25, 27) were provisionally identified as "Kunimasu". The lack of dark dorsal spots agreed with previous reports on body coloration of Kunimasu (Jordan and Hubbs 1925; Hikita 1962). On the other hand, 21 male specimens collected in September (FAKU 99370–99383, 99385, 99387–99390, 99392, 99393), agreeing with the description of mature male Himemasu given by Jordan and Hubbs (1925) (non-black body with dark spots on dorsum), were provisionally identified as "Himemasu". The remaining 58 specimens (FAKU 98737, 98738, 98742, 99167–99189, 99328, 99384, 99386, 99391, 99394, 99396–99401, 99435–99445, 99452–99456, 99461–99463, YFTC 21, 26) plus 12 black-bodied "Uki-yo" specimens collected in January and February (FAKU 98741, YFTC 1–11), which may have included both Kunimasu and Himemasu, were left unidentified at this stage. Internal morphology, including numbers of gill rakers and pyloric caeca, was not used for

identification purposes, due to these characters not being available in some specimens. Examples of Kunimasu (9) and Himemasu (20) (FAKU97747–97766) from Lake Saiko, examined by Nakabo et al. (2011), plus 30 “pure” Himemasu specimens from Lake Akan (FAKU97921–97950), for which no documented record of Kunimasu stocking exists, were also examined. Muscle tissues were obtained from all but three specimens and preserved in 99.5% ethanol. For the latter three, either a liver tissue sample or a fin clip was used.

Microsatellite genotyping. Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Germany) or the Wizard Genomic DNA Purification Kit (Promega, USA), both following the manufacturers’ protocols. Five microsatellite loci isolated from sockeye salmon (One102, One108, One110, One114, One115; Olsen et al. 2000) were amplified via the polymerase chain reaction (PCR) using fluorescent-labeled primers detected by an ABI-310 genetic analyzer. PCR protocols followed Nakabo et al. (2011).

Statistical analysis - population structure. The population structure was explored using a Bayesian-based clustering method implemented in the program STRUCTURE 2.3 (Pritchard et al. 2000), wherein individuals are clustered on the basis of genotype into K randomly interbreeding groups by minimizing departures from Hardy-Weinberg equilibrium and linkage disequilibrium within the groups. Ten replicates for each pre-defined K = 1 between 10 were obtained with runs of 100,000 burn-in and 200,000 Markov Chain Monte Carlo (MCMC) iterations, applying the admixture model with correlated alleles. Specimens were pooled into two sampling locations (Lakes Saiko and Akan) for use in the LOCPRIOR model (Hubisz et al. 2009), which uses the sampling locations of specimens to assist in clustering. To determine the most likely value of K, the Evanno et al. (2005) method implemented in the program STRUCTURE HARVESTER (Earl 2009), which determines the second-order rate of change in the distribution of L (K), was used. CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007) was used to summarize parameters across 10 replicate runs and DISTRUCT ver.1.1 (Rosenberg 2004), to produce the corresponding graphical output. Each specimen was assigned to one or

more inferred groups on the basis of that individual's proportion of membership or proportion of the genome of individuals originating from those groups (*q*i). As proposed by Vähä and Primmer (2006), specimens were assigned to one group if *q*i > 0.90 (purebred individuals) or jointly to two or more groups if the proportion of membership to each was *q*i < 0.90 (hybrid individuals), that value having been widely adopted in other studies seeking to detect hybrids (e.g., Burgarella et al. 2009; Sanz et al. 2009).

A second Bayesian method, NEWHYBRIDS 1.0 (Anderson and Thompson 2002), which aims specifically at detecting hybrids between species, was also applied. In NEWHYBRIDS, each individual's genotype frequency class (genetic class) is inferred, thus providing a posterior probability (*Q*) for reflecting the level of certainty of an individual belonging to a certain genetic class (for example, F1, backcross, purebred.) Six genetic classes were estimated, viz. pure Kunimasu, pure Himemasu, F1, F2, backcross of F1 with pure Kunimasu and that with pure Himemasu, with a burn-in of 50,000 iterations followed by a sampling period of 50,000 iterations, assuming no prior allele frequencies and a uniform prior distribution for diversity at each locus. The z option was used for Himemasu from Lake Akan to specify in advance that they were of known origin and belonged to the "pure Himemasu" class, thereby allowing their use in estimating allele frequencies among Himemasu.

Levels of genetic differentiation between populations were evaluated by pairwise F_{ST}, calculated using ARLEQUIN ver. 3.5 (Excoffier et al. 2005).

Statistical analysis - genetic diversity and bottleneck effects. The observed and expected heterozygosity, linkage disequilibrium between all combinations of locus pairs within populations and deviations from Hardy-Weinberg equilibrium were calculated using ARLEQUIN ver. 3.5 (Excoffier et al. 2005). Allelic diversity was assessed as allelic richness (calculated in FSTAT ver. 2.9.3.2 (Goudet 2002)), being a measure of the number of alleles per population corrected for sample size. Three measures were used to determine whether or not the Kunimasu population had experienced a recent bottleneck. Mode shifts in allele frequencies

within populations and increased heterozygosity relative to that expected under the mutation-drift equilibrium (heterozygosity excess) were tested in BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996), assuming an infinite alleles model of mutation (IAM). BOTTLENECK employs the Wilcoxon signed-rank test to detect heterozygosity excess. The statistic *M*, which measures allelic "vacancies" within the allelic range, was calculated according to Garza and Williamson (2001). Differences in expected heterozygosity, allelic richness and *M* values among populations were tested using Kruskal-Wallis test in R language version 2.11.1. (R developmental core team 2010). Where appropriate, all statistical tests were conducted at family-wise significance level of $\alpha = 0.05$, using Holm' s sequential Bonferroni adjustment (Holm 1979).

Results

Genetic divergence and introgressive hybridization. All five loci used in this study were polymorphic, the total number of alleles per locus ranging from 11 (One102) to 17 (One110) and allelic richness ranging from 8.9 (One102) to 12.3 (One115). Significant linkage disequilibrium was observed between one pair of loci (One102 and One115) in Himemasu from Lake Akan among a total of 30 tests (initial $\alpha = 0.0017$). There were no significant departures from Hardy-Weinberg equilibrium after sequential Bonferroni adjustment (initial $\alpha = 0.0033$). Allele frequencies for the five loci are presented in Table S2.

In the STRUCTURE analysis, the most likely number of clusters was shown to be $K = 2$, according to the Evanno et al. (2005) method (Fig. 1). Each specimen was then assigned to one or other of the clusters based on *q*i. All 33 specimens of provisionally-identified "Kunimasu" were assigned to one cluster, whereas all 41 specimens of "Himemasu" from Lake Saiko were to the other, together with 30 specimens of indigenous Himemasu from Lake Akan (Fig. 2). Therefore, we regarded the former cluster as "genetically-assigned Kunimasu" and the latter as "genetically-assigned Himemasu". Note that "genetically-assigned Kunimasu" and

179 "genetically-assigned Himemasu" are hereafter referred to simply as Kunimasu and Himemasu,
180 respectively. Of the 70 unidentified specimens, 38 were assigned to Kunimasu and the
181 remainder to Himemasu (Fig. 2). All but two specimens (FAKU 97737 and 97767) displayed *q*i
182 > 0.90, indicating a high probability of the former being purebred individuals (Vähä and
183 Primmer 2006). Although the other two specimens were determined as possible hybrids, they
184 were assigned to Kunimasu on the basis of their dominant Kunimasu genome, their *q*i being
185 0.87 and 0.73. The result of the NEWHYBRIDS analysis was consistent with that of the
186 STRUCTURE analysis (not shown). Using a *Q* value of 0.50 as a threshold for assigning
187 specimens to a specific genetic class, no specimens were assigned to any of the hybrid classes
188 (F1, F2 and two backcrosses). Even when a threshold value of > 0.50 for the sum of the *Q*
189 values of all hybrid classes was applied, no hybrid specimens were found.
190 Moderate but highly significant genetic differentiations (F_{ST}) were observed between
191 Kunimasu and the two populations (Lakes Saiko and Akan) of Himemasu, such differentiation
192 not being apparent between the latter (Table 2).
193 **Genetic diversity and bottleneck effects.** Within-population genetic summary statistics
194 for Kunimasu and the two Himemasu populations are presented in Table 1. Average expected
195 heterozygosity and allelic richness were higher in Kunimasu than in Himemasu, both being
196 significantly different among populations (Kruskal-Wallis test: expected heterozygosity $\chi^2 = 9.5$,
197 $P = 0.009$, allelic richness $\chi^2 = 9.4$, $P = 0.009$). No mode shift in allele frequency distributions
198 were detected (Fig. S1). Kunimasu had an excess of heterozygosity relative to that expected at
199 mutation-drift equilibrium (Table 1), but this effect was not statistically significant ($P = 0.031$,
200 initial $\alpha = 0.017$). The *M* values were all above the conservative significance threshold of $M =$
201 0.68 (Garza and Williamson 2001) and did not differ significantly among populations
202 (Kruskal-Wallis test, $\chi^2 = 0.67$, $P = 0.715$).
203
204 **Discussion**

205 **Genetic divergence and introgressive hybridization.** The introduction of non-indigenous
206 species and habitat modification can result in introgressive hybridization between otherwise
207 allopatric species, which may lead to the extinction of participating species through the loss of
208 distinct gene pools and local adaptive features, and breakdown of co-adapted gene complexes
209 (Allendorf and Waples 1995; Rhymer and Simberloff 1996; Seehausen 2006). Previous studies
210 examining introgressive hybridization in salmonid fishes following secondary contact have
211 described various findings. In Crane Praire Reservoir in the Northwestern US, Matala et al.
212 (2008) observed genetic distinctiveness and highly restricted, if any, gene flow between wild
213 red band trout (*Oncorhynchus mykiss gairdeneri*) and hatchery rainbow trout (*Oncorhynchus*
214 *mykiss irideus*), despite extensive stocking of the latter. In Lake Biwa, Japan, Kuwahara et al.
215 (2012) recently reported a moderate level of introgression of the *Oncorynchus masou* subsp.
216 (Biwamasu) [*Oncorhynchus* sp. sensu Nakabo (2009)] genome into lake-run *Oncorhychus*
217 *masou ishikawae* (Amago), the latter being absent from Lake Biwa before stocking was
218 initiated in 1970. Bettles et al. (2005) reported ongoing introgressive hybridization between
219 cutthroat trout (*Oncorhynchus clarki clarki*) and rainbow trout (*O. mykiss irideus*) in 13
220 streams on Vancouver Island, Canada, the degree of which varied among streams from F1
221 dominance to the formation of a hybrid swarm.

222 The present results indicated highly restricted introgressive hybridization between
223 Kunimasu and Himemasu, despite the large scale introduction of the latter into both Lakes
224 Tazawa and Saiko. According to the STRUCTURE analysis based on five microsatellite loci, all
225 but two (FAKU 97737 and 97767) of a total of 144 specimens were assigned to Kunimasu and
226 Himemasu clusters with high probability. Although the remaining two specimens were
227 suggested as being hybrids, the Kunimasu genome was dominant in both of them, indicating
228 that they were not F1 progeny. Furthermore, the NEWHYBRIDS and STRUCTURE analysis
229 results were consistent, indicating an absence of hybrids having either a Kunimasu or
230 Himemasu ancestor within two generations (F1, F2, backcross of F1 with pure Kunimasu and

231 those with pure Himemasu) in the specimens sampled, leading to our conclusion that
232 introgressive hybridization between Kunimasu and Himemasu has been rare, resulting in the
233 persistence of distinct gene pools of the two species in Lake Saiko. Notwithstanding, some
234 traces of past introgressive hybridization were identified, although it is not clear whether this
235 occurred (pre-1940) in Lake Tazawa or subsequently in Lake Saiko.

236 Vähä and Primmer's (2006) simulation study using STRUCTURE proposed the use of an
237 arbitrary threshold of qi = 0.90 for separating hybrids from purebreds, despite showing at that
238 time that such a value tended to overestimate the proportion of hybrids. Application of a more
239 stringent threshold (qi = 0.80) in the present study did not affect the result significantly, one
240 specimen still being assigned to hybrids. The STRUCTURE analysis also tends to overestimate
241 the proportion of hybrids when the number of loci used is small (Vähä and Primmer 2006), but
242 this effect should have been compensated for by the inclusion of reference population allele
243 frequency information (Himemasu from Lake Akan) and the relatively large genetic divergence
244 between Kunimasu and Himemasu (Vähä and Primmer 2006). Nevertheless, further analysis
245 with an increased number of loci may further clarify the relationship between the two species.

246 Spatial and temporal segregation of spawning have both been reported as primary factors
247 underlying the divergence of sympatric species or populations of the genus *Oncorhynchus* (e.g.,
248 Taylor et al. 1997; Fillatre et al. 2003; Lin et al. 2008; Pavey et al. 2010). Although the ecology
249 and life history of Kunimasu remain largely unknown, Nakabo (2011) and Nakabo et al. (2011)
250 suggested that Kunimasu in Lake Tazawa had become adapted to a particularly low water
251 temperature spawning ground, occurring in deeper water than for any other species of
252 *Oncorhynchus.* Nakabo et al. (2011) also noted that their Kunimasu specimens had been
253 collected while or just after spawning in March and April, in accordance with the putative main
254 spawning season of Kunimasu in Lake Tazawa (February) (Nakabo 2011), whereas Himemasu
255 generally spawn in autumn, as described by, for example, Tokui (1959, 1961). Apparently, both
256 spatial and temporal segregation of spawning of Kunimasu and Himemasu in Lake Saiko has

257 led to their reproductive isolation.

258 Genetic differentiation (F_{ST}) between Kunimasu and Himemasu (Table 2) was comparable
259 to that previously reported among populations of *O. nerka*, inferred from microsatellite DNA
260 variations. Genetic differentiation between Kunimasu and Himemasu was greater than that
261 observed among Japanese Himemasu stocks (pairwise F_{ST} = -0.008–0.032; Yamamoto et al.
262 2011), but less than that between sockeye salmon from the Abira River, Hokkaido [derived from
263 Himemasu in Lake Shikotsu, Hokkaido (Urawa et al. 1999)] and other sockeye salmon
264 populations from various localities around the Pacific rim (pairwise F_{ST} = 0.199–0.310;
265 Beacham et al. 2006), suggesting that Kunimasu represents one of a number of populations of
266 kokanee. Nevertheless, Kunimasu have evolved unique morphological and ecological
267 characteristics within *O. nerka*, presumably because of the unique environment of Lake Tazawa,
268 being on the periphery of the range of *O. nerka*, such uniqueness contributing to the
269 reproductive isolation between Kunimasu and Himemasu (see above).

270 Kunimasu has been reported by several authors as having a uniformly black body in life
271 (Akitaken Suisanshikenjo 1907; Oshima 1940; Nakabo et al. 2011), lacking dark dorsal spots
272 (Jordan and Hubbs 1925; Hikita 1962). In contrast, Oshima (1941) described Kunimasu as
273 having small indistinct dark spots on the dorsum (on the basis of one male specimen). In the
274 present study, all specimens of provisionally-identified “Kunimasu”, which had a black body
275 without dark dorsal spots, were subsequently genetically assigned to the Kunimasu cluster,
276 according to the STRUCTURE analysis. On the other hand, the genetic assignment of the 70
277 previously unidentified specimens yielded mixed results, 38 and 32 specimens being assigned to
278 the Kunimasu and Himemasu clusters, respectively. The former included 10 specimens with a
279 black body with dark dorsal spots (FAKU 99442, 99444, 99452, 99454, 99562, 99463, YFTC 6,
280 8, 9, 11), 19 with a black body without dark spots (FAKU 98737, 98738, 98741, 98742,
281 99396–99400, 99443, 99453, 99461, YFTC 1–5, 7, 10), seven with a non-black body with dark
282 spots (FAKU 99187, 99189, 99386, 99435, 99437, 99439, YFTC 26) and two with a non-black

283 body without dark spots (FAKU99182, 99328). Such variations in body coloration, previously
284 unreported, need to be considered in relation to life history phase. A detailed morphological
285 description of Kunimasu will be considered in a forthcoming publication.

286 ***Genetic diversity and population bottleneck.*** Small isolated or recently bottlenecked
287 populations are most likely to suffer from an increased chance of extinction or reduced adaptive
288 potential due to reduced genetic diversity, as well as from other deterministic and/or stochastic
289 effects (Frankham et al. 1999, 2002). Although the extant Kunimasu population was established
290 recently from a single founding event involving ca. 100,000 eggs, the genetic diversity of
291 Kunimasu, measured by expected heterozygosity and allelic richness, was no less than that of
292 Himemasu from Lakes Saiko and Akan (this study), or from other Japanese (Yamamoto et al.
293 2011) and North American kokanee populations (Young et al. 2004). In addition, the Kunimasu
294 population did not show any evidence of having been subjected to a bottleneck, according to
295 three measures used herein to determine the likelihood of such, i.e., a mode shift in allele
296 frequency distributions, heterozygosity excess and a reduced value for *M*. A mode shift of
297 allele frequency distributions in a bottlenecked population occurs such that the proportion of
298 alleles at an intermediate frequency exceeds the proportion of rare alleles, because rare alleles
299 are lost rapidly as the result of small population size (Luikart et al. 1998). The observed
300 heterozygosity in bottlenecked population would be increased relative to the heterozygosity
301 expected under mutation-drift equilibrium estimated from the observed number of alleles, as
302 allelic diversity is reduced faster than heterozygosity during a bottleneck. The statistic *M*, which
303 measures allelic “vacancies” within the allelic range, can be expected to be reduced in
304 bottlenecked populations as the latter have more “vacancies” than non-bottlenecked populations.
305 However, each of these genetic tests failed to detect any signs of a bottleneck in the Kunimasu
306 population.

307 In conclusion, the present Kunimasu population in Lake Saiko is not considered to be
308 seriously endangered, at least by introgressive hybridization and reduced genetic diversity.

309 However, the rate of introgressive hybridization may differ in different situations. In fact, two
310 Kunimasu-like specimens collected in Lake Motosu in December 2010, to which ca. 100,000
311 eyed eggs of Kunimasu were introduced in 1935 (Sugiyama 2000), have both been shown to be
312 hybrids of Kunimasu and Himemasu, the Himemasu genome being dominant, following a
313 preliminary genetic analysis by STRUCTURE. Environmental changes in Lake Saiko have the
314 potential to intensify introgressive hybridization between Kunimasu and Himemasu, as evident
315 already for several sympatric species pairs (Seehausen 2006), some of which have resulted in
316 breakdown into a a hybrid swarm. Clearly, therefore, future conservation efforts for Kunimasu
317 in Lake Saiko should include periodic monitoring of the genetic properties of the species.
318
319 **Acknowledgments** We thank Yasuaki Miura, Y. Watanabe, Hisashi Miura, Hiroshi Miura,
320 Manabu Miura, K. Mitsui, T. Aoyagi, N. Kaji, M. Ohashi, S. Sasaki, M. Nozu and N. Sakai for
321 their help in collecting specimens, and A. Tokairin and K. Kimura for their assistance in the
322 laboratory. We also thank G. S. Hardy for his critical reading of the manuscript. This study was
323 partly supported by the Core Stage Backup Research Fund of Kyoto University and a
324 Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science
325 (24370036).
326
327 **References**
328 Akitaken Suisanshikenjo (1907) Kunimasu jinkofuka shiken. Akitaken Suisanshikenjo
329 Jigyohokoku 1907:35–55
330 Allendorf FW, Waples RS (1995) Conservation and genetics of salmonid fishes. In: Avise JC,
331 Hamrick JL (eds) Conservation Genetics: Case Histories from Nature. Chapman and Hall,
332 New York, pp 238–280
333 Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids
334 using multilocus genetic data. Genetics 160:1217–1229

335 Beacham TD, McIntosh B, MacConnachie C, Miller, KM, Withler RE, Varnavskaya, N (2006)
336 Pacific rim population structure of sockeye salmon as determined from microsatellite
337 analysis. Trans Am Fish Soc 135:174–187
338 Bettles CM, Docker MF, Dufour B, Heath DD (2005) Hybridization dynamics between
339 sympatric species of trout: loss of reproductive isolation. J Evol Biol 18:1220–1233
340 Burgarella C, Lorenzo Z, Jabbour-Zahab R, Lumaret R, Guichoux E, Petit RJ, Soto A, Gil L
341 (2009) Detection of hybrids in nature: application to oaks (*Quercus suber* and *Q. ilex*).
342 Heredity 102:442–452
343 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent
344 population bottlenecks from allele frequency data. Genetics 144:2001–2014
345 Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for
346 visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet
347 Res. doi:10.1007/s12686-011-9548-7
348 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the
349 software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
350 Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for
351 population genetics data analysis. Evol Bioinform Online 1:47–50
352 Fillatre EK, Etherton P, Heath DD (2003) Bimodal run distribution in a northern population of
353 sockeye salmon (*Oncorhynchus nerka*): life history and genetic analysis on a temporal scale.
354 Mol Ecol 12:1793–1805
355 Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge
356 University Press, Cambridge
357 Frankham R, Lees K, Montgomery ME, England PR, Lowe EH, Briscoe DA (1999) Do
358 population size bottlenecks reduce evolutionary potential? Anim Conserv 2:255–260
359 Garza JC, Williamson EG (2001) Detection of reduction in population size using data from
360 microsatellite loci. Mol Ecol 10:305–318

361 Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices
362 (version 2.9.3.2). http://www.unil.ch/izea/softwares/fstat.html. Accessed 3 May 2012
363 Hikita T (1962) Ecological and morphological studies of the genus *Oncorhynchus* (Salmonidae)
364 with particular consideration on phylogeny. Sci Rep Hokkaido Salmon Hatchery 17:1–97
365 Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–70
366 Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with
367 the assistance of sample group information. Mol Ecol Resour 9:1322–1332
368 Jakobsson M, Rosenberg NA (2007) *CLUMPP*: a cluster matching and permutation program for
369 dealing with label switching and multimodality in analysis of population structure.
370 Bioinformatics 23:1801–1806
371 Jordan DS, Hubbs CL (1925) Record of fishes obtained by David Starr Jordan in Japan, 1922.
372 Mem Carnegie Mus 10:93–346, pls 5–12
373 Kuwahara M, Takahashi H, Kikko T, Kurumi S, Iguchi K (2012) Introgression of
374 *Oncorhynchus masou* subsp. (Biwa salmon) genome into lake-run *O. m. ishikawae* (Amago
375 salmon) introduced into Lake Biwa, Japan. Ichthyol Res. doi:10.1007/s10228–011–0270–y
376 Lin J, Quinn TP, Hilborn R, Hauser L (2008) Fine-scale differentiation between sockeye salmon
377 ecotypes and the effect of phenotype on straying. Heredity 101:341–350
378 Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency
379 distributions provides a test for recent population bottlenecks. J Hered 89:238–247
380 Matala AP, Marx S, Wise TG (2008) A genetically distinct wild redband trout (*Oncorhynchus*
381 *mykiss gairdneri*) population in Crane Prairie Reservoir, Oregon, persists despite extensive
382 stocking of hatchery rainbow trout (*O. m. irideus*). Conserv Genet 9:1643–1652
383 Nakabo T (2009) Zoogeography of Taiwanese Fishes. Korean J. Ichthyol. 21:311–321
384 Nakabo T (2011) ‘‘Kunimasu’’ *Oncorhynchus kawamurae* (Pisces:Salmonidae), 70 years after
385 extinction in Lake Tazawa, Akita Prefecture, Japan. Taxa Proc Jpn Soc Syst Zool
386 30:31–54

387 Nakabo T, Nakayama K, Muto N, Miyazawa M (2011) *Oncorhynchus kawamurae*
388 ‘‘Kunimasu,’’ a deepwater trout, discovered in Lake Saiko, 70 years after extinction in the
389 original habitat, Lake Tazawa, Japan. Ichthyol Res 58:180–183
390 Olsen JB, Wilson SL, Kretschmer EJ, Jones KC, Seeb JE (2000) Characterization of 14
391 tetranucleotide microsatellite loci derived from sockeye salmon. Mol Ecol 9:2185–2187
392 Oshima M (1940) Sekitsuidobutsu taikei: sakana (Vertebrata: Pisces). Sanseido, Tokyo
393 Oshima M (1941) Sake-masu zoku no kishu Tazawako no Kunimasu ni tsuite. Nippon
394 Gakujutsu Kyokai Hokoku 16:254–259
395 Pavey SA, Nielsen JL, Hamon TR (2010) Recent ecological divergence despite migration in
396 sockeye salmon (*Oncorhynchus nerka*). Evolution 64:1773–1783
397 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus
398 genotype data. Genetics 155:945–959
399 R developmental core team (2010) R: a language and environment for statistical computing. R
400 Foundation for Statistical Computing, Vienna
401 Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. Annu Rev Ecol
402 Syst 27:83–109
403 Rosenberg NA (2004) *Distruct*: a program for the graphical display of population structure. Mol
404 Ecol Notes 4:137–138
405 Sanz N, Araguas RM, Ferna´ndez R, Vera M, Garcı´a-Marı´n J (2009) Efficiency of markers
406 and methods for detecting hybrids and introgression in stocked populations. Conserv
407 Genet 10:225–236
408 Seehausen O (2006) Conservation: losing biodiversity by reverse speciation. Curr Biol
409 16:R334–R337
410 Sugiyama H (2000) Tazawako maboroshi no sakana: Kunimasu hyakka. Akita Sakigake
411 Shimpo, Akita

412 Taylor EB, Harvey S, Pollard S, Volpe J (1997) Postglacial genetic differentiation of
413 reproductive ecotypes of kokanee *Oncorhynchus nerka* in Okanagan Lake, British
414 Columbia. Mol Ecol 6:503–517
415 Tokui T (1959) Studies on the kokanee salmon in Lake Towada, northern Honshu, Japan (II).
416 Climate and hydrologic factors. Sci Rep Hokkaido Salmon Hatchery 14:169–192
417 Tokui T (1961) Studies on the kokanee salmon IV. Spawning migration of the kokanee salmon
418 in Lake Shikotsu, Hokkaido, Japan. Sci Rep Hokkaido Salmon Hatchery 16:127–136
419 Urawa S, Ban M, Furukawa M, Suzuki T, Kaeriyama M (1999) Progressive technologies for
420 artificial production of anadromous sockeye salmon in Japan. Bull. Tohoku Natl. Fish. Res.
421 Inst. 62:141–150
422 Utter F (2001) Patterns of subspecific anthropogenic introgression in two salmonid genera. Rev
423 Fish Biol Fisheries 10:265–279
424 Vähä JP, Primmer CR (2006) Efficiency of model-based Bayesian methods for detecting hybrid
425 individuals under different hybridization scenarios and with different numbers of loci. Mol
426 Ecol 15:63–72
427 Yamamoto S, Kitamura S, Sakano H, Morita K (2011) Genetic structure and diversity of
428 Japanese kokanee *Oncorhynchus nerka* stocks as revealed by microsatellite and
429 mitochondrial DNA markers. J Fish Biol 79:1340–1349
430 Young SF, Downen MR, Shaklee JB (2004) Microsatellite DNA data indicate distinct native
431 populations of kokanee, *Oncorhynchus nerka*, persist in the Lake Sammamish Basin,
432 Washington. Environ Biol Fishes 69:63–79
433
434

435 **Table 1** Sample size (*n*), number of alleles, allelic richness (*A*), expected and observed
436 heterozygosity (H_E, H_O), expected heterozygosity under mutation-drift equilibrium (H_{Eq}) and *M*
437 values (*M*) in five microsatellite loci of Kunimasu *Oncorhynchus kawamurae* and two
438 populations of Himemasu *O. nerka*

		Ok-LS	On-LS	On-LA
One102				
	n	71	73	30
	No. of alleles	11	5	5
	A	10.005	4.559	4.967
	H_E	0.853	0.522	0.486
	H_O	0.859	0.562	0.433
	H_{Eq}	0.736	0.482	0.547
	M	0.917	0.833	0.833
One108				
	n	71	73	30
	No. of alleles	11	10	8
	A	10.175	8.588	7.932
	H_E	0.891	0.768	0.742
	H_O	0.789	0.726	0.633
	H_{Eq}	0.734	0.707	0.711
	M	0.786	0.667	0.533
One110				
	n	71	73	30
	No. of alleles	11	11	10
	A	9.805	7.837	9.766

	H_E	0.882	0.618	0.584
	H_O	0.859	0.603	0.533
	H_{Eq}	0.735	0.733	0.773
	M	0.524	0.786	0.625
One114				
	n	71	72	29
	No. of alleles	11	9	8
	A	10.257	8.615	8.000
	H_E	0.885	0.819	0.825
	H_O	0.831	0.792	0.655
	H_{Eq}	0.734	0.675	0.713
	M	0.917	1.000	0.889
One115				
	n	71	73	30
	No. of alleles	12	11	9
	A	10.672	9.792	8.899
	H_E	0.884	0.621	0.567
	H_O	0.845	0.630	0.567
	H_{Eq}	0.757	0.735	0.743
	M	0.800	0.917	0.900
Mean H_E		0.879	0.670	0.641
Mean number of alleles		11.2	9.2	8.0
Mean *A*		10.183	7.878	7.913
Mean *M*		0.789	0.840	0.756
Heterozygosity excess (*P*-value)		0.031	1.000	0.219

439 *Ok-LS* Kunimasu, *Oncorhynchus kawamurae*; *On-LS* Himemasu, *O. nerka*, from Lake Saiko;

441

442

443

444 **Table 2** Pairwise *F*st values between populations (below diagonal) and associated *P*-values
445 (above diagonal) inferred from five microsatellite loci
446

447

	Ok-LS	On-LS	On-LA
Ok-LS		0.0000	0.0000
On-LS	0.1342		0.5387
On-LA	0.1416	-0.0005	

449

450 *Ok-LS* Kunimasu, *Oncorhynchus kawamurae*, *n* = 71; *On-LS* Himemasu, *O. nerka*, from Lake
451 Saiko, *n* = 73; *On-LA* Himemasu from Lake Akan, *n* = 30

452

453

Figure legends

Fig. 1 Average delta *K* over 10 replicate runs of STRUCTURE for a given number of clusters (*K*) for the specimens examined

Fig. 2 Model-based clustering analysis of Kunimasu *Oncorhynchus kawamurae* and two populations of Himemasu *O. nerka* computed by STRUCTURE version 2.3 with $K = 2$. Vertical bars represent individuals and probability of assignment to each cluster (black; Kunimasu, white; Himemasu). Arrows indicate putative hybrids. Provisional identification of each specimen shown at bottom.

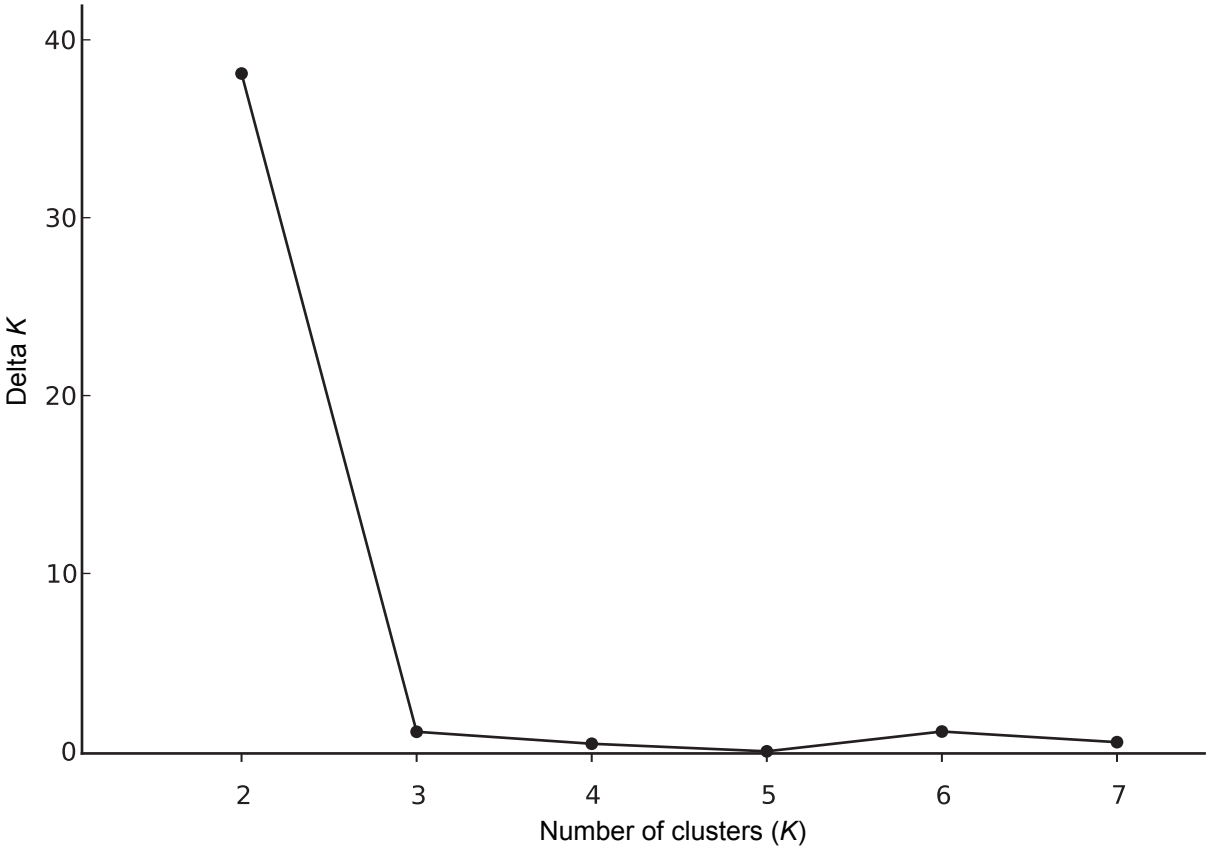

Fig. 1 Average delta *K* over 10 replicate runs of STRUCTURE for a given number of clusters (*K*) for the specimens examined

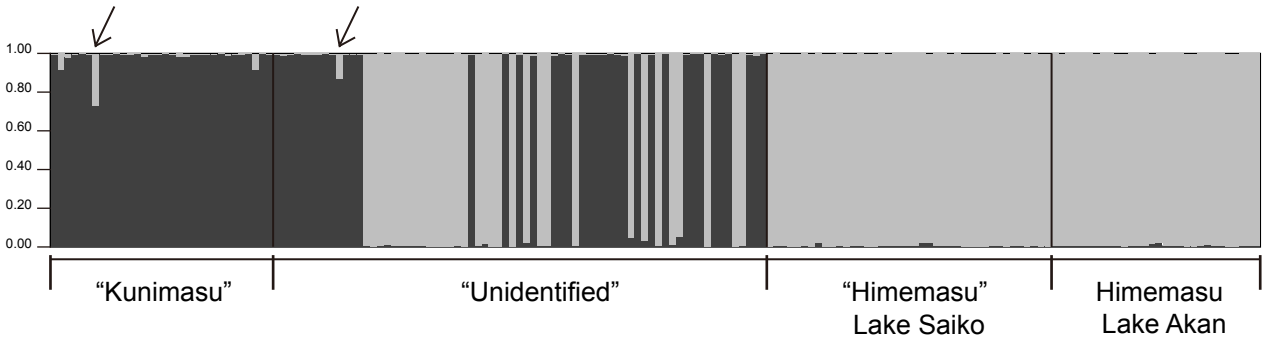

Fig. 2 Model-based clustering analysis of Kunimasu *Oncorhynchus kawamurae* and two populations of Himemasu *O. nerka* computed by STRUCTURE version 2.3 with $K = 2$. Vertical bars represent individuals and its probability of assignment to each cluster (black; Kunimasu, white; Himemasu). Arrows indicate putative hybrids. Provisional identification of each specimen shown at bottom.

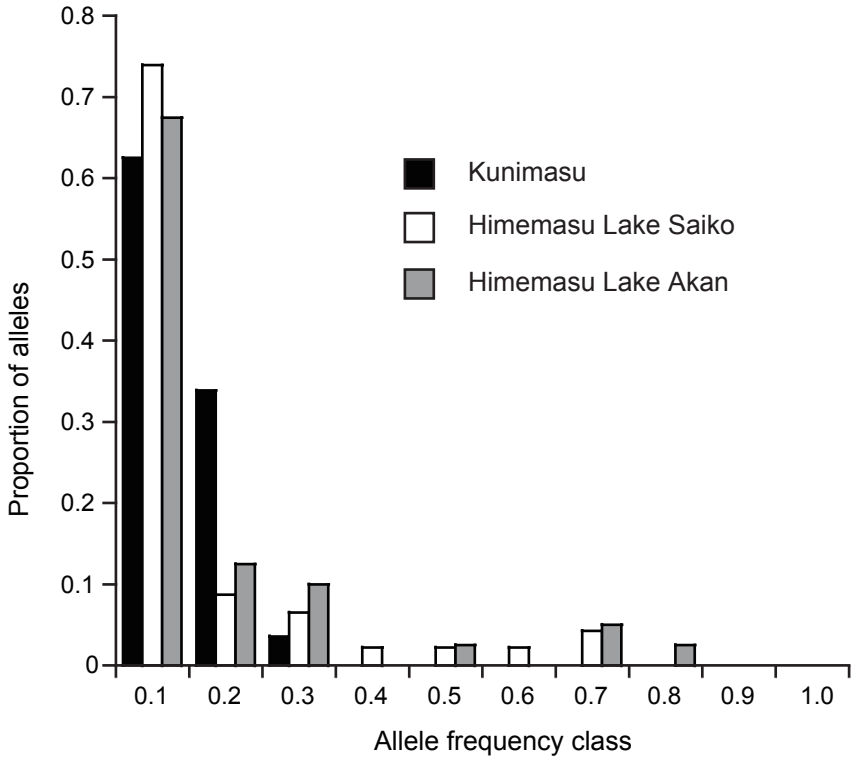

Fig. S1 Allele frequency distribution of Kunimasu *Oncorhynchus kawamurae* and two populations of Himemasu *O. nerka*

Table S1 Sampling locality, date, method, body color, sex, provisional identification, genetic assignment and catalogue number of each specimen examined

Locality	Date	Method	Body color	sex	Provisional identification	Genetic assignment	Catalogue number
Lake Saiko	20100306	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU97736
Lake Saiko	20100306	Gill net	b	Male	"Kunimasu"	Kunimasu*	FAKU97737
Lake Saiko	20100319	Gill net	b	Female	"Kunimasu"	Kunimasu	FAKU97742
Lake Saiko	20100319	Gill net	b	Female	"Kunimasu"	Kunimasu	FAKU97743
Lake Saiko	20100319	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU97744
Lake Saiko	20100319	Gill net	b	Female	"Kunimasu"	Kunimasu	FAKU97745
Lake Saiko	20100404	Gill net	c	Unknown	"Himemasu"	Himemasu	FAKU97747
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97748
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97749
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97750
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97751
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97752
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97753
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97754
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97755
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97756
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97757
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97758
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97759
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97760
Lake Saiko	20100404	Gill net	d	Unknown	"Himemasu"	Himemasu	FAKU97761
Lake Saiko	20100404	Gill net	d	Male	"Himemasu"	Himemasu	FAKU97762
Lake Saiko	20100404	Gill net	c	Male	"Himemasu"	Himemasu	FAKU97763
Lake Saiko	20100404	Gill net	d	Female	"Himemasu"	Himemasu	FAKU97764
Lake Saiko	20100403	Angling	d	Unknown	"Himemasu"	Himemasu	FAKU97765
Lake Saiko	20100404	Gill net	c	Male	"Himemasu"	Himemasu	FAKU97766
Lake Saiko	20100404	Gill net	b	Male	"Kunimasu"	Kunimasu*	FAKU97767
Lake Saiko	20100306	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU97768
Lake Saiko	20100306	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU97769
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97921
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97922
Lake Akan	20100511	Gill net	c	Female	Pure Himemasu	Himemasu	FAKU97923
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97924
Lake Akan	20100511	Gill net	c	Female	Pure Himemasu	Himemasu	FAKU97925
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97926
Lake Akan	20100511	Gill net	c	Female	Pure Himemasu	Himemasu	FAKU97927
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97928
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97929
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97930
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97931
Lake Akan	20100511	Gill net	c	Female	Pure Himemasu	Himemasu	FAKU97932
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97933
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97934
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97935
Lake Akan	20100511	Gill net	c	Female	Pure Himemasu	Himemasu	FAKU97936
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97937
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97938
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97939
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97940
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97941
Lake Akan	20100511	Gill net	d	Female	Pure Himemasu	Himemasu	FAKU97942
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97943
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97944
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97945
Lake Akan	20100511	Gill net	d	Male	Pure Himemasu	Himemasu	FAKU97946
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97947
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97948
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97949
Lake Akan	20100511	Gill net	c	Male	Pure Himemasu	Himemasu	FAKU97950
Lake Saiko	20101219	Angling	b	Female	"Unidentified"	Kunimasu	FAKU98737
Lake Saiko	20101219	Angling	b	Male	"Unidentified"	Kunimasu	FAKU98738
Lake Saiko	20110106	Beached	b	Male	"Unidentified"	Kunimasu	FAKU98741
Lake Saiko	20101229	Angling	b	Female	"Unidentified"	Kunimasu	FAKU98742
Lake Saiko	20110302	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98834
Lake Saiko	20110302	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98835
Lake Saiko	20110302	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98836

Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98837
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98838
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98839
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98840
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98841
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98842
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98843
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98844
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98845
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98846
Lake Saiko	20110303	Gill net	b	Male	"Kunimasu"	Kunimasu	FAKU98847
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99167
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99168
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99169
Lake Saiko	20110620	Angling	d	Unknown	"Unidentified"	Himemasu	FAKU99170
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99171
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99172
Lake Saiko	20110620	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99173
Lake Saiko	20110621	Angling	d	Unknown	"Unidentified"	Himemasu	FAKU99174
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99175
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99176
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99177
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99178
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99179
Lake Saiko	20110622	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99180
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99181
Lake Saiko	20110623	Angling	d	Unknown	"Unidentified"	Kunimasu	FAKU99182
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99183
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99184
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99185
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99186
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Kunimasu	FAKU99187
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99188
Lake Saiko	20110623	Angling	c	Unknown	"Unidentified"	Kunimasu	FAKU99189
Lake Saiko	20110426	Angling	d	Female	"Unidentified"	Kunimasu	FAKU99328
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99370
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99371
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99372
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99373
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99374
Lake Saiko	20110908	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99375
Lake Saiko	20110910	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99376
Lake Saiko	20110907	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99377
Lake Saiko	20110907	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99378
Lake Saiko	20110907	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99379
Lake Saiko	20110910	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99380
Lake Saiko	20110910	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99381
Lake Saiko	20110910	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99382
Lake Saiko	20110910	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99383
Lake Saiko	20110910	Gill net	d	Male	"Unidentified"	Himemasu	FAKU99384
Lake Saiko	20110911	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99385
Lake Saiko	20110911	Gill net	c	Female	"Unidentified"	Kunimasu	FAKU99386
Lake Saiko	20110911	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99387
Lake Saiko	20110911	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99388
Lake Saiko	20110911	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99389
Lake Saiko	20110911	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99390
Lake Saiko	20110907	Gill net	d	Male	"Unidentified"	Himemasu	FAKU99391
Lake Saiko	20110907	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99392
Lake Saiko	20110907	Gill net	c	Male	"Himemasu"	Himemasu	FAKU99393
Lake Saiko	20110907	Gill net	c	Female	"Unidentified"	Himemasu	FAKU99394
Lake Saiko	20111001	Angling	b	Female	"Unidentified"	Kunimasu	FAKU99396
Lake Saiko	20111001	Angling	b	Male	"Unidentified"	Kunimasu	FAKU99397
Lake Saiko	20111001	Angling	b	Female	"Unidentified"	Kunimasu	FAKU99398
Lake Saiko	20111001	Angling	b	Female	"Unidentified"	Kunimasu	FAKU99399
Lake Saiko	20111001	Angling	b	Male	"Unidentified"	Kunimasu	FAKU99400
Lake Saiko	20111001	Angling	a	Male	"Unidentified"	Himemasu	FAKU99401
Lake Saiko	20111002	Angling	c	Male	"Unidentified"	Kunimasu	FAKU99435
Lake Saiko	20111002	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99436
Lake Saiko	20111002	Angling	c	Female	"Unidentified"	Kunimasu	FAKU99437
Lake Saiko	20111002	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99438
Lake Saiko	20111002	Angling	c	Male	"Unidentified"	Kunimasu	FAKU99439

Lake Saiko	20111002	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99440
Lake Saiko	20111004	Angling	c	Male	"Unidentified"	Himemasu	FAKU99441
Lake Saiko	20111004	Angling	a	Male	"Unidentified"	Kunimasu	FAKU99442
Lake Saiko	20111016	Angling	b	Male	"Unidentified"	Kunimasu	FAKU99443
Lake Saiko	20111016	Angling	a	Male	"Unidentified"	Kunimasu	FAKU99444
Lake Saiko	20111016	Angling	a	Male	"Unidentified"	Himemasu	FAKU99445
Lake Saiko	20111020	Angling	a	Male	"Unidentified"	Kunimasu	FAKU99452
Lake Saiko	20111020	Angling	b	Female	"Unidentified"	Kunimasu	FAKU99453
Lake Saiko	20111020	Angling	a	Female	"Unidentified"	Kunimasu	FAKU99454
Lake Saiko	20111017	Angling	c	Unknown	"Unidentified"	Himemasu	FAKU99455
Lake Saiko	20111017	Angling	c	Female	"Unidentified"	Himemasu	FAKU99456
Lake Saiko	20111109	Angling	b	Male	"Unidentified"	Kunimasu	FAKU99461
Lake Saiko	20111109	Angling	a	Male	"Unidentified"	Kunimasu	FAKU99462
Lake Saiko	20111109	Angling	a	Male	"Unidentified"	Kunimasu	FAKU99463
Lake Saiko	20110114	Beached	b	Female	"Unidentified"	Kunimasu	YFTC1
Lake Saiko	20110115	Beached	b	Unknown	"Unidentified"	Kunimasu	YFTC2
Lake Saiko	21110119	Beached	b	Male	"Unidentified"	Kunimasu	YFTC3
Lake Saiko	20110130	Beached	b	Female	"Unidentified"	Kunimasu	YFTC4
Lake Saiko	20110216	Beached	b	Female	"Unidentified"	Kunimasu	YFTC5
Lake Saiko	20110218	Beached	a	Female	"Unidentified"	Kunimasu	YFTC6
Lake Saiko	20110218	Beached	b	Female	"Unidentified"	Kunimasu	YFTC7
Lake Saiko	20110218	Beached	a	Female	"Unidentified"	Kunimasu	YFTC8
Lake Saiko	20110218	Beached	a	Female	"Unidentified"	Kunimasu	YFTC9
Lake Saiko	20110218	Beached	b	Male	"Unidentified"	Kunimasu	YFTC10
Lake Saiko	20110224	Beached	a	Female	"Unidentified"	Kunimasu	YFTC11
Lake Saiko	20110302	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC14
Lake Saiko	20110302	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC15
Lake Saiko	20110324	Gill net	b	Female	"Kunimasu"	Kunimasu	YFTC18
Lake Saiko	20110325	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC19
Lake Saiko	20110325	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC20
Lake Saiko	20110325	Gill net	c	Female	"Unidentified"	Himemasu	YFTC21
Lake Saiko	20110325	Gill net	b	Female	"Kunimasu"	Kunimasu	YFTC22
Lake Saiko	20110325	Gill net	b	Female	"Kunimasu"	Kunimasu	YFTC23
Lake Saiko	20110325	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC24
Lake Saiko	20110325	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC25
Lake Saiko	20110325	Gill net	c	Male	"Unidentified"	Kunimasu	YFTC26
Lake Saiko	20110331	Gill net	b	Male	"Kunimasu"	Kunimasu	YFTC27

a black with dark spots, *b* black without dark spots, *c* not black with dark spots, *d* not black without dark spots
* Putative hybrids

Table S2 Allele frequencies for five microsatellite loci of Kunimasu *Oncorhynchus kawamurae* and two populations of Himemasu *O. nerka*

	Ok–LS	On–LS	On–LA
One102			
n	71	73	30
218	0.014	–	–
222	0.028	–	–
226	0.134	–	–
230	0.106	0.226	0.15
234	0.021	0.075	0.067
238	0.141	0.651	0.7
242	0.148	0.034	0.067
246	0.268	–	–
250	0.028	0.014	0.017
254	0.092	–	–
258	–	–	–
262	0.021	–	–
One108			
n	71	73	30
179	0.007	–	–
183	0.049	–	–
187	0.148	–	–
191	0.134	–	–
195	0.141	0.11	0.1
199	0.07	0.144	0.217
203	0.077	0.068	0.15
207	0.134	0.425	0.433
211	0.12	0.116	0.033
215	0.099	0.034	0.033
219	–	–	–
223	–	–	–
227	–	0.068	0.017
231	0.021	–	–
235	–	0.014	–
239	–	–	–
243	–	–	–
247	–	0.014	–
251	–	0.007	0.017
One110			
n	71	73	30
207	0.007	–	–
211	–	–	–

215	0.007	–	–
219	–	–	–
223	–	–	–
227	–	–	–
231	0.07	0.562	0.6
235	–	0.007	0.017
239	–	0.007	–
243	–	–	–
247	–	0.014	–
251	0.085	–	0.017
255	0.176	–	–
259	0.141	0.062	0.017
263	0.085	0.041	0.017
267	0.183	0.021	0.017
271	0.085	0.014	0.033
275	0.085	0.007	0.017
279	–	0.253	0.25
283	–	0.014	–
287	0.077	–	–
291	–	–	0.017
One114			
n	71	72	29
227	–	0.097	0.052
231	–	0.069	0.19
235	–	0.028	0.052
239	0.113	0.042	–
243	0.077	0.306	0.259
247	0.162	0.222	0.259
251	0.183	0.146	0.103
255	0.077	0.069	0.034
259	0.028	0.021	0.052
263	0.049	–	–
267	0.141	–	–
271	0.092	–	–
275	0.07	–	–
279	–	–	–
283	0.007	–	–
One115			
n	71	73	30
177	0.007	–	–
181	0.07	–	–
185	0.007	–	–
189	0.028	–	–

193	0.148	0.007	0.017
197	0.056	0.048	0.067
201	0.127	0.055	0.017
205	0.099	0.034	0.033
209	0.106	0.048	–
213	0.07	0.027	0.083
217	0.211	0.603	0.65
221	–	0.089	0.067
225	–	0.034	0.017
229	–	0.027	0.05
233	0.07	–	–
237	–	0.027	–

Ok-LS Kunimasu, *Oncorhynchus kawamurae*; *On-LS* Himemasu, *O. nerka*, from Lake Saiko; *On-LA* Himemasu from Lake Akan